Tropical Journal of Pharmaceutical Research June 2022; 21 (6): 1189-1193
ISSN: 1596-5996 (print); 1596-9827 (electronic)

Available online at http://www.tjpr.org
http://dx.doi.org/10.4314/tjpr.v21i6.8

Original Research Article

Lactobacillus Kimchicus-fermented *Ficus caria* fruits and leaves extract as an inhibitor of α-MSH-mediated melanogenesis in B16F10 mouse melanoma cells

Ji Hyung Kim[1], Hwa Jun Cha[2]*
[1]SJ Natural Inc, Yong-in, [2]Osan University, Department of Beauty and Cosmetics, Osan, South Korea

For correspondence: **Email: hjcha@osan.ac.kr*

Sent for review: 21 June 2021 *Revised accepted: 26 May 2022*

Abstract

Purpose: *To explore the anti-melanogenetic effects of Lactobacillus Kimchicus-fermented Ficus caria fruits and leaves extracts (KFFE) in B16F10 mouse melanoma cells.*
Methods: *The anti-melanogenetic effects of Lactobacillus Kimchicus-fermented Ficus caria fruit and leaf extracts (KFFE) were assessed using melanin contents assay and tyrosinase expression and activity assay in α-MSH-treated B16F10 mouse melanoma cells.*
Results: *The KFFE reduced α-MSH-mediated melanin synthesis in B16F10 mouse melanoma cells, while the tyrosinase mRNA expression was down-regulated by KFFE in B16F10 mouse melanoma cells ($p < 0.05$). Furthermore, cellular tyrosinase activity was downregulated by KFFE in B16F10 mouse melanoma cells ($p < 0.05$).*
Conclusion: *KFFE functions to repress melanin synthesis and thus, can potentially be used as a cosmetic ingredient.*

Keyword: *Lactobacillus kimchicus, Ficus caria, Melanogenesis, B16F10, Tyrosinase*

Tropical Journal of Pharmaceutical Research is indexed by Science Citation Index (SciSearch), Scopus, International Pharmaceutical Abstract, Chemical Abstracts, Embase, Index Copernicus, EBSCO, African Index Medicus, JournalSeek, Journal Citation Reports/Science Edition, Directory of Open Access Journals (DOAJ), African Journal Online, Bioline International, Open-J-Gate and Pharmacy Abstracts

INTRODUCTION

Various external stimuli continuously assail the body. However, almost all the external stimuli are blocked by the epidermis (the outermost layer of skin) [1]. Ultraviolet radiation, one of these external stimuli, can penetrate the structural block layer and induce photo-aging [2,3]. Exposure to ultraviolet radiation causes burns, inflammation, inhibition of the immune response, and damage to skin connective tissues. Persistent ultraviolet exposure destroys skin structure and ultimately causes photo-aging and skin cancer [4]. Thus, the epidermis prevents UV radiation from passing through the skin by synthesizing melanin [5,6]. The melanin is synthesized in melanosome of melanocytes and is induced by various factors, such as ultraviolet light, cytokines, growth factors and hormones, and through various mechanisms [7,8]. When melanogenesis (synthesis of melanin) is initiated by such factors, tyrosinase is expressed which then sequentially catalyzes the oxidation of L-tyrosine (one of the amino acids, to melanin in melanosome) [9,10]. Since the synthesized melanin is distributed to keratinocytes, skin is

able to resist ultraviolet radiation [11,12]. However, abnormal melanogenesis causes cosmetic disorders, such as melasma and freckles, and non-uniform pigmentation which causes skin to age [13]. Therefore, the cosmetics industry seeks to address the issue of skin aging by regulating melanogenesis [14,15]. Currently, whitening agents used in the cosmetics field include arbutin and kojic acid, which inhibit tyrosinase activity [14,16,17]. However, since these whitening agents are beset by problems of stability and safety, only a limited amount is used. Thus, development of whitening materials using products from natural source is required [15]. This study therefore seeks to investigate *Lactobacillus Kimchicus*-fermented *Ficus caria* fruit and leaf extract as a possible whitening agent by testing its ability to inhibit α-MSH-mediated melanogenesis in mouse melanoma cells.

EXPERIMENTAL

Cell culture

The B16F10 mouse melanoma cells were purchased from Korea cell line bank (KCLB, Korea) and grown in 10 % fetal bovine serum (FBS; Sigma-Aldrich, USA), penicillin 100 units/mL and streptomycin 100 µg/mL (Gibco, USA) - containing Dulbecco's modified Eagle's medium (DMEM; Gibco) at 37 ^{o}C in 5 % CO_2 in a humidified condition.

Preparation of *Lactobacillus Kimchicus*-fermented *Ficus caria* fruit and leaf extracts (KFFE)

Ficus caria fruits and leaves (ratio of fruits and leaves weight = 1:1) were washed and dried in a dry oven at 60 ^{o}C (Daehan Science, Korea). The dried *Ficus caria* fruits and leaves were pulverized, and the contents were extracted using 70 % ethanol with over 20 kHz ultrasonic waves for 2 h. After the extraction, the ethanoic extract was filtered with Whatman filter paper No. 2 (GE Healthcare Life Science, USA), and ethanol was removed from the extract of *Ficus caria* fruits and leaves using a rotary evaporator (EYELA, Japan). Then, *Lactobacillus Kimchicus* was incubated in the extract of *Ficus caria* fruits and leaves and incubated for 72 h. After fermentation, KFFE was fully dried with a lyophilizer (Ilshin, Korea). The dehydrated extract was dissolved in dimethyl sulfoxide (DMSO).

Cell viability assay

Cell viability was determined with 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. The B16F10 cells (3 x 10^3) were seeded in each well of the 96 well plate and grown to 80 % confluence. In addition, KFFE was added to the culture for 48 h, and then 0.5 mg/mL MTT was added (Sigma-Aldrich). Then the MTT formazan was dissolved in DMSO and the amount of MTT formazan was measured by a Microplate Reader (Molecular Device, USA) at 595 nm wavelength.

Evaluation of melanin contents

All B16F10 cells (3 x 10^3) were seeded in each well of the 96-well plate and grown to 80 % confluence. Then, KFFE and α-MSH (100 ng/mL) were added to the culture for 48 h. After the experiments, all samples were lysed using 1 N sodium hydroxide solution (Biopure) at 95 ^{o}C for 15 min. Melanin content was determined using optical density at 450 nm. The melanin content was normalized using total protein amount. The total protein of each lysate was measured by BCA Assay (Thermo Fisher Scientific, USA).

Intracellular tyrosinase activity assay

The B16F10 cells were lysed with Triton X-100 lysis buffer (1 % Triton X-100, 150 mM NaCl, 50 mM HEPES (pH 7.5) and 5 mM EDTA). The supernatant was separated from precipitate by centrifugation and reacted with 2 mM L-DOPA (Sigma-Aldrich) for 30 min at 37 ^{o}C. After the reaction, optical density was measured using a Microplate reader (Molecular Device) at 450 nm. The total protein content of each lysate was measured by BCA Assay (Thermo Fisher Scientific).

Quantitative real time-polymerase chain reaction (qRT-PCR)

Total RNAs were isolated using TRIzol (Invitrogen, USA). The purity and concentration of the total RNA was measured using a micro volume spectrophotometer (Maestrogen Inc., USA). The cDNAs of each sample were synthesized using reverse transcriptase (Qiagen, Germany) according to the manufacturer's protocol. Quantitative real-time PCR was performed using Real-Time PCR (Applied Biosystems, USA).

Tyrosinase specific forward primer: 5'-CAAGTACAGGGATCGGCCAAC-3'; Tyrosinase specific reverse primer: 5'-GGTGCATT GGCTTCTGGGTAA-3'. The PCR was performed using qRT-PCR premix (Solis Biodyne, Spain). All samples were normalized with β-actin.

Statistical analysis

All experiments were repeated three times and data presented as mean ± standard deviation (SD). Student's *t*-test was analyzed for statistical significance using Excel 2018 (Microsoft, USA), and statistical outcomes was considered significant at $p < 0.05$.

RESULTS

Cell viability of KFFE in B16F10 mouse melanoma cells

To reveal whether KFFE was cytotoxic to B16F10 melanoma cells, B16F10 cells were treated with KFFE at the indicated concentration for 48 h and then MTT assay was performed in KFFE exposed cells. As shown in Figure 1, treatment with KFFE caused no significant change in cell viability up to 50 μg/mL. Therefore, the following experiments on the anti-melanogenesis effects of KFFE were performed within 50 μg/mL that had no cytotoxicity.

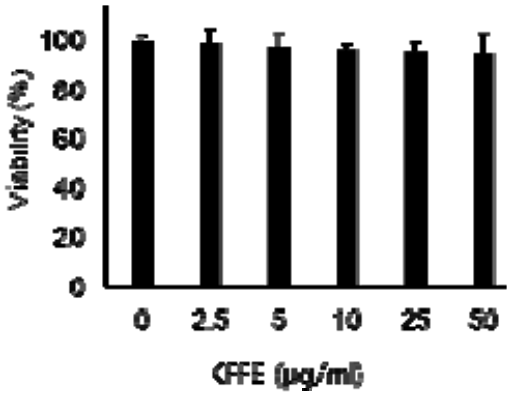

Figure 1: Cytotoxic effect of extracts of KFFE in B16F10 mouse melanoma cells

KFFE decreased melanin contents in B16F10 mouse melanoma cells

To investigate the effects of KFFE on melanin contents, after treatment with α-MSH (100 ng/mL) and KFFE simultaneously, the synthesized melanin amount was compared in KFFE treated B16F10 cells and α-MSH only treated B16F10 cells as a negative control (Figure 2). The KFFE reduced the melanin content in a concentration dependent manner. The melanin concentration was greatly inhibited at 50 μg/mL KFFE. Since melanin was produced by tyrosinase in melanosome, the tyrosinase activity was measured in subsequent experiments [8].

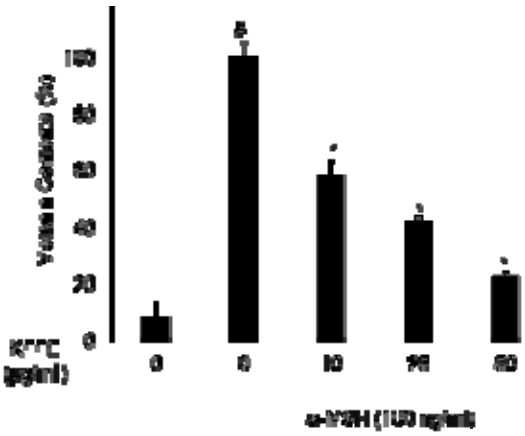

Figure 2: Effect of KFFE on melanin contents in B16F10 cells (melanin content was measured by optical density at 450 nm). *$P < 0.05$ compared with α-MSH only treated B16F10 cells

KFFE decreased tyrosinase activity and expression in B16F10 mouse melanoma cell

To verify cellular tyrosinase activity in KFFE-treated B16F10 cells, B16F10 cells were treated with 50 μg/mL KFFE and α-MSH (100 ng/mL). Cellular tyrosinase activity was decreased from 100 % to 53.24 % by 50 μg/mL KFFE in 100 ng/mL α-MSH treated B16F10 cells (Figure 3). Additionally, it demonstrated that the expression level of tyrosinase mRNA was decreased from 100 to 37.24 % by 50 μg/mL LFFE in 100 ng/mL α-MSH treated B16F10 cells (Figure 4). There was no significant difference between control and KFFE treated cells ($p > 0.5$). Cytotoxicity was presented as a percentage compared to control.

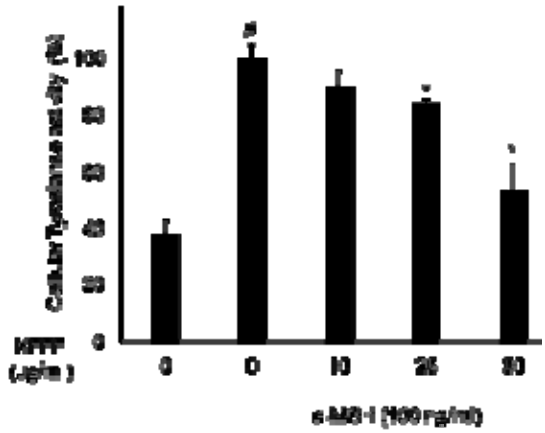

Figure 3: Effect of KFFE on cellular tyrosianse activity in B16F10 cells. *$P < 0.05$ compared with α-MSH only treated B16F10 cells

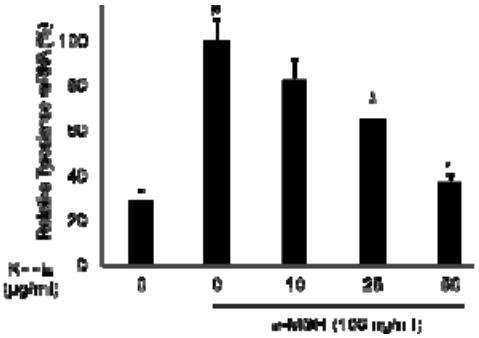

Figure 4: Effect of KFFE on the expression of tyrosinase mRNA in B16F10 cells. $^{\#}P < 0.05$ compared with non-treated B16F10 cells, $^{*}p < 0.05$ compared with α-MSH only treated B16F10 cells

DISCUSSION

Melanin synthesis is regulated by the expression of melanogenesis related enzymes [17-19]. In the early stage, tyrosinase converts L-tyrosine to L-DOPA, and then melanin is finally synthesized by tyrosinase, TRP-1 and TRP-2 from L-DOPA [19]. Therefore, in this study, the expression level of tyrosinase was first ascertained. Tyrosinase is a key regulator in melanin biosynthesis, using qRT-PCR. At the concentration of 50 μg/mL expression of tyrosinase, an essential enzyme for the production of melanin, was inhibited by KFFE. In particular, 50 μg/mL KFFE inhibited cellular tyrosinase activity 46.76 % in the α-MSH treatment group. Sequentially, melanin content was decreased by 50 μg/mL KFFE to 37.24 % compared to the α-MSH treatment group (100 %).

According to the results, this study postulates that KFFE inhibits the formation of melanin as well as the expression of melanin-related enzyme, and thus, has a whitening effect. Therefore, it can be used as an ingredient for whitening functional cosmetics.

CONCLUSION

The KFFE exerts an anti-melanogenesis effect in B16F10 cells by inhibiting cellular tyrosinase activity and expression of tyrosinase mRNA. Its anti-melanogenesis activity provides a pharmacological and cosmetic basis for the development of natural compounds for hypopigmentation therapy.

DECLARATIONS

Acknowledgement

This study was carried out with the support of R & D Program for Forest Science Technology (Project no. 2020271A00-2020-AD03) provided by Korea Forest Service (Korea Forestry Promotion Institute). This research was also supported by Osan University in 2022.

Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them. Hwa Jun Cha designed all the experiments and revised the paper. Ji Hyung Kim and Hwa Jun Cha performed the experiments and wrote the paper.

Open Access

REFERENCES

1. *Rinnerthaler M, Bischof J, Streubel MK, Trost A, Richter K. Oxidative stress in aging human skin. Biomolecules 2015; 5: 545-589.*
2. *Farage MA, Miller KW, Elsner P, Maibach HI. Characteristics of the aging skin. Adv Wound Care (New Rochelle) 2013; 2: 5-10.*
3. *Zhang S, Duan E. Fighting against skin aging: The way from bench to bedside. Cell Transplant 2018; 27: 729-738.*
4. *Pandel R, Poljšak B, Godic A, Dahmane R. Skin photoaging and the role of antioxidants in its prevention. ISRN Dermatol 2013; 2013: 930164.*
5. *Brenner M, Hearing VJ. The protective role of melanin against UV damage in human skin. Photochem Photobiol 2008; 84: 539-549.*
6. *D'Orazio J, Jarrett S, Amaro-Ortiz A, Scott T. UV radiation and the skin. Int J Mol Sci 2013; 14: 12222-12248.*
7. *Videira IF, Moura DF, Magina S. Mechanisms regulating melanogenesis. An Bras Dermatol 2013; 88(1): 76-83.*
8. *Yuan XH, Jin ZH. Paracrine regulation of melanogenesis. Br J Dermatol 2018; 178:632-639.*

9. D'Mello SA, Finlay GJ, Baguley BC, Askarian-Amiri ME. Signaling pathways in melanogenesis. Int J Mol Sci 2016; 17: E1144.
10. Rzepka Z, Buszman E, Beberok A, Wrześniok D. From tyrosine to melanin: Signaling pathways and factors regulating melanogenesis. Postepy Hig Med Dosw (Online) 2016; 70: 695-708.
11. Cichorek M, Wachulska M, Stasiewicz A, Tymińska A. Skin melanocytes: biology and development. Postepy Dermatol Alergol 2013; 30: 30-41.
12. Maranduca MA, Branisteanu D, Serban DN, Branisteanu DC, Stoleriu G, Manolache N, Serban IL. Synthesis and physiological implications of melanic pigments. Oncol Lett 2019; 17: 4183-4187.
13. Bastonini E, Kovacs D, Picardo M. Skin pigmentation and pigmentary disorders: focus on epidermal/dermal cross-talk. Ann Dermatol 2016; 28: 279-289.
14. Couteau C, Coiffard L. Overview of skin whitening agents: Drugs and cosmetic products. Cosmetics 2016; 3: 27.
15. Smit N, Vicanova J, Pavel S. The hunt for natural skin whitening agents. Int J Mol Sci 2009; 10: 5326-5349.
16. Desmedt B, Courselle P, De Beer JO, Rogiers V, Grosber M, Deconinck E, De Paepe K. Overview of skin whitening agents with an insight into the illegal cosmetic market in Europe. J Eur Acad Dermatol Venereol 2016; 30: 943-950.
17. Zolghadri S, Bahrami A, Hassan Khan MT, Munoz-Munoz J, Garcia-Molina F, Garcia-Canovas F, Saboury AA. A comprehensive review on tyrosinase inhibitors. J Enzyme Inhib Med Chem 2019; 34: 279-309.
18. Pillaiyar T, Manickam M, Jung SH. Recent development of signaling pathways inhibitors of melanogenesis. Cell Signal 2017; 40: 99-115.
19. Tief K, Hahne M, Schmidt A, Beermann F. Tyrosinase, the key enzyme in melanin synthesis, is expressed in murine brain. Eur J Biochem 1996; 241: 12-16.